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Acute and Sub-acute oral toxicity studies of Deedan-A Unani drug in Albino rats

Showkat A. Dar[1,2*], Khalid Ghazanfar[1,2], Seema Akbar[1], Akbar Masood[2], Tazeen Nazir[1], Khalid M. Siddiqui[3], Pawan Kumar[3]

[1]Regional Research Institute of Unani Medicine, Srinagar, J&K, India.
[2]Department of Biochemistry, University of Kashmir, Srinagar, J&K, India.
[3]Central Council for Research in Unani Medicine, New Delhi, India.

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ABSTRACT

Deedan is a very effective compound formulation of Unani System of medicine used for the treatment of worm infestation. The objective of this study was to investigate the Acute and Sub-acute toxicity of Deedan in Albino rats of both the sexes. In the acute toxicity study, Deedan was administered orally at the limit dose of 2000mg/kg b.w. to both male and female rats, and the animals were then observed individually 30 minutes, 4 hour post-dosing, and at least twice daily for next 14 days. In the Sub-acute toxicity study a limit dose of 1000 mg/kg body weight was administered orally in a single bolus everyday for 28 days. The rats were observed daily during the period of study, and sacrificed on the 29th day. Observation parameters of the animals included a comparative evaluation of general appearance/behaviour, morbidity/mortality, body weights, food/water consumption, haematology, biochemistry and histopathology of major organs of treated and control groups. There was no mortality, morbidity, or cage-side/laboratory findings of any adverse health effect in the treated animals in comparison to their respective controls in both toxicity studies. Deedan was thus found to be free of any toxic effects under the conditions of these studies.

INTRODUCTION

The Unani System of Medicine in the past few decades has emerged as a most popular alternative mode of treatment (Razzack, 2006) and acquired a very sound position in the Indian System of Medicine. It's researchers have not only been treating patients suffering from various ailments including life threatening ones through the use of its products but also taking a lead in research. These products are now gaining interest at international level, too. This interest is serious because the existing preparation of allopathic origin are known to have adverse reactions which themselves can be sometimes life threatening. However for Unani drugs to be accepted by the international community they have to satisfy the three important criteria of Quality, Efficacy and Safety when tested through contemporary scientific tools. There are about more than a thousand Unani drugs in clinical use available in different shapes but their use in relatively limited. This is because little effort, if any, has been made towards a systematic understanding of their toxic potential. Toxicological study is the key for survival of herbal formulation across the world (Wal et al., 2011; Debbie et al., 2012). Since patent regime are varied opportunities can be created for bringing the products by providing this information. Once the safety profile of these Unani Medicinal Formulations (UMFs) are defined, they would stand a better chance to compete with other drugs (e.g., Chinese Herbal Formulations), and they would be better accepted by the International Regulatory Agencies. Deedan is polyherbal formulation used against various human endoparasites like *Trichuris trichiura* (whipworm), *Enterobius vermicularis* (pinworm) and *Giardiasis* (CCRUM, 2007). The constituents of the Deedan is shown in table 1. Efficacy of Deedan is well defined but little data is available pertaining to its toxicity as per the standard guidelines. In this study an effort was made to produce a toxicity profile of Deedan drugs following standard guideline acceptable internationally. Dosage of Deedan in Humans is 1 capsule of 500mg twice a day orally with water for 7 days.

* *Corresponding Author*
Email: seemaakbar@hotmail.com

This amounts to about 14.4mg/kg/day in human subjects. In order to assess the potential toxic effects of Deedan, it was administered to young, healthy rats at different dose level in the two different acute and sub-acute safety studies.

Table 1: Constituents of Deedan.

Ingredient	English / Scientific Name	Amount
i. Maghz-e-karanjwa bonducella	Physic Nut, Mulacca Bean/Caesalpinia	71.5mg
ii. Habbul Neel	Indigo, Pharbitis Nil /Indigofera tinctoria	71.5mg
iii. Tukhm-e-Hanzal	Colocynth / *Citrullus colocynthis*	71.5mg
iv. Turbud	Turpeth / *Operculina turpethum*	71.5mg
v. Kamila	Indian Kamila / Mallotus philippensis	71.5mg
vi. Palaspapra *monosperma*	Bengal Kino Tree, Flame of the forest / *Butea*	71.5mg
vii. Afsanteen absinthium	Worm Wood, Southern Wood / Artemisia	71.5mg
	Total	**500mg.**

MATERIAL AND METHODS

Test item

The work was carried on Deedan supplied by CRI, Hyderabad India. The batch number of received drug was U269 and the date of manufacture was May 2011. The Deedan was encapsulated and the powder was mixed with RO water to make the suspension.

Experimental Animals

The experimental animals (young, healthy Albino rats of Wistar Strain) were procured from IIIM, Jammu. These rats were kept in the animal house and were observed during the quarantine and acclimatization period (Capdevila et al., 2007). A veterinary examination was conducted on the rats prior to and at the end of the acclimatization and quarantine period of 14 days. The rats were housed under standard environmental condition at a temperature of 20 – 25^{0}C with 12:12 hour dark and light cycle. The rats were provided pelleted feed procured from Pranov Agro Industries, New Delhi and filtered/sterilized water (Aqua Guard KENT RO). The experimental work was carried on the guidelines set by CPCSEA, India. The use of animals in the study was approved by the IAEC, RRIUM, Srinagar which is registered with the CPCSEA, India.

Acute Oral Toxicity Test

The acute oral toxicity test was carried as per OECD guideline (OECD, 2008). Adult male and female Albino rats of 100-150 grams body weight (8-12 weeks age) were used. Five animals of each sex were used in each of the two treated and control groups (total 20 animals). All the rats were weighted to record their initial body weight before dosing, and on the 7th and 14th day of doing (before sacrifice). The test substance was administered orally by using feeding canula. Rats were fasted overnight, but allowed water *ad libitum* prior to feeding of drug. The Group I and II being the male and female Controls were given RO water in comparable volumes to the treated animals (Vehicle only). Animals of Group III (males) and IV (females) were treated with the test item Deedan at the dose level of 2000 mg per kg body weight. All the rats were observed individually for any adverse signs and behavioral changes at an interval of 30 minutes, 4 hour post dosing and twice daily for 14 days. During the study period of 14 days, the feed and water consumption / rat / 24 hours were recorded at initial stage, after 1 week of dosing and before the sacrifice. Finally on the 15th day all the animals were sacrificed by withdrawing blood in a syringe from the dorsal vena cava after opening the abdomen under ISOFLURANE anaesthesia. 2ml of blood was added to EDTA vacutainer for the study of Haemato-logical parameters and 3 ml blood was added to Red tap vacutainer containing the clotting activators. The clotted blood was centrifuged and the serum was separated for the study of Bio-chemistry parameters. The internal organs were examined macro-scopically for the visualization of morphological changes, if any.

Sub-acute Oral Toxicity Test

The sub-acute oral toxicity was conducted in accordance to the OECD guideline (OECD, 2008). The rats were randomly divided into 4 groups each group consisted of 5 rats. The Group I and II being the male and female Controls were orally treated with RO water (Vehicle). The Group III and IV being the experimental male and female rats were orally treated with Deedan at the dose of 1000mg/kg body weight for 28 days daily. All the animals were closely observed for the first 1 and 4 hours of dosing to examine any adverse toxic signs, behavioural changes etc. The body weight of the rats was evaluated weekly. Feed and water consumption/ rat/24 hours were recorded before dosing and then weekly upto 4 weeks. On the 29th day, after over-night fast, all the animals were sacrificed by withdrawing blood in a syringe from the dorsal vena cava after opening the abdomen under ISOFLURANE anaesthesia. All the animals were dissected to check macroscopic morphology of the body organs. The organs such as liver, lung, kidney, adrenal gland, pancreas, spleen, brain, ovary/testes and heart were collected to determine the relative organ weight followed by grossing for the collection of tissues for Histopathological studies.

Biochemistry Parameters

Biochemical parameters were studied in serum obtained after centrifugation of blood at 2000 RPM for 15 minutes on the day of the rat sacrifice. Biochemical parameters were determined on fully automatic biochemistry analyzer (XL600 TRANSASIA) using ERBA kits. Liver function tests- aspartate aminotransferase AST, alanine aminotransferase ALT, alkaline phosphotase ALP, Total bilirubin, total protein, albumin, kidney function tests- blood urea, uric acid and other metabolic function tests such glucose, Cholesterol and Triglycerides were estimated.

Haematological Parameters

Haematological parameters were analyzed in freshly collected blood in blue top vacutainer containing EDTA

anticoagulant. The blood was gently mixed with the EDTA anticoagulant coated on the tube walls. Haematological parameters were determined on fully automatic haematological analyzer (Sysmex XT2000iV Sysmex Corporation Japan). Haematological parameters such as Haemoglobin conc. WBC count, RBC count, haematocrit value, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration, Mean Corpuscular Haemoglobin, Platelate count, differential leukocyte count – Neutrophil %, Lymphocyte %, Monocyte %, Eosinophil % and basophil %, and Reticulocyte count were studied.

Histopathology

Tissue samples were collected from the organs of control as well as treated male / female rats of the Sub-acute study. The tissue collected from the organs such as liver, lung, kidney, adrenal gland, pancreas, spleen, brain, ovary/testes and heart ware numbered for identification and then transferred to tissue cassettes (SS) to enable fixation in 10 % formalin for 36-48 hours followed by the tissue processing which was carried on Automatic tissue processor Model No1020 (LIECA make Germany). The tissue processing included dehydration in graded isopropyl alcohol, clearing in xylene I & II, impregnation in paraffin wax and finally tissue blocks was prepared on paraffin block making Model No1150H+C (LIECA make Germany). Section cutting of tissue blocks was done using microtome (YARCO) to the thickness of 4-5 microns.

The tissue sections were fixed on the slide by heat technique following by the staining by Haematoxylin and Eosin stain. The staining was carried on Automatic slide stainer (THERMO MAKE Germany) haematoxylin and eosin staining. After staining the tissue section were mounted with DPX to prevent any damage to the stained tissue. The stained tissue sections were examined under microscope 40x and 100x objective to check the adverse effects of drug on cell morphology as well as on the cell organelles.

Statistical analysis

All results are expressed as mean ± standard deviation. Comparison of all results on body weight, food & water consumption, haematological value, biochemical values, were performed using one way analysis of variance (ANOVA) method using statistical software SPSS version 16.0. Probability of 0.05 or small ($p \leq 0.05$) was used as the criterion of significance.

RESULTS

Acute Oral Toxicity Test

Group Mean body weight

The rats treated with Deedan at the dose of 2000 mg/kg of body weight were found to grow and gain body weight normally and there was no treatment related change found in the body weight gains. Table 2 shows the body weight of rats in acute toxicity study.

Table 2: Body weight of rats in acute toxicity.

Group	Body weight (g)		
	0^{th} Day	7^{th} day	14^{th} Day
Male (Treated)	121±6.4	145±6.1	177±12.3
Male (Control)	125±8.2	139±10.3	168 ±7.1
Female (Treated)	146±4.7	161±4.3	171±3.61
Female (Control)	116± 6.0	122±6.2	129± 6.9

The values are expressed as mean ± SD. n=5 in each group

Average Feed and Water consumption

The average feed and water consumption of both treated groups were found to be unaffected by the Deedan treatment as there were no significant changes in the average feed consumption and water consumption when compared with the respective controls as shown in table (3).

Table 3: Average feed and water consumption of rats.

Average feed consumption (g/day)		Average water consumption (ml/day)	
Male	Female	Male	Female
Control	18.5 ±2.4	17.6±2.5	25.2±2.6	26.1 ±2.2
Treated	18.3±1.5	18.2±0.5	27.3±1.5	27.1 ±1.5

The values are expressed as mean ± SD. n=5 in each group. $^{*}p<0.05$ as compared with the controls at the same time (one-way ANOVA)

Biochemistry Parameters

The results of Biochemical parameters did not show any significant change in the levels when compared with the controls as shown in table 4.

Haematology Parameters

The results of Haematological parameters of the treated males and females did not show any significant change in the values when compared with the respective controls. Table 5 shows the haematological parameters of rats.

Group Mean body weight

The rats treated with Deedan at the dose of 1000 mg/kg of body weight were found to grow and gain body weight normally and there was no any adverse effect found in the body weight gains. Table 6 shows the body weight of rats in sub-acute toxicity study.

Average Feed and Water consumption

The average feed and water consumption of both treated groups was found to be unaffected by the Deedan treatment as there were no significant changes in the average feed consumption and water consumption when compared with the respective controls as shown in table (7).

Biochemistry Parameters

The results of Biochemical parameters did not show any significant change in the values when compared with the controls except a moderate reduction in triglycerides in both sexes which indicates the Deedan reduces the triglyceride level in the blood circulation as shown in table (8).

Table 4: Biochemical parameters of rats in acute toxicity study.

Parameter	Male		Female	
	Control	Treated	Control	Treated
Liver function tests				
ALT (IU/L)	88.3 ±9.6	87.8± 8.5	84.9±13.8	83.2±12.4
AST (IU/L)	154 ±15.3	154±21.8	166±19.6	160±20.6
ALP (IU/L)	191±28.7	189±15.3	192±15.1	187 ±14.9
Total Bilirubin (mg/dl)	0.1±0.07	0.1± 0.01	0.12±0.07	0.11±0.01
Total Protein (g/dl)	7.7±0.37	7.96 ± 0.25	8.02±0.61	7.98 ±0.54
Albumin (g/dl)	4.5±0.09	4.5± 0.30	4.28±0.66	4.52±0.19
Kidney function tests				
Urea (mg/dl)	52.3±4.3	51.2±2.2	51.1±10.2	52.2± 4.9
Uric acid (mg/dl)	2.98±0.52	3.04±0.3	3.65±0.8	3.72 ± 1
Metabolic function tests				
Glucose (mg/dl)	80.3±6.5	80.7±17.6	82.5 ±13	86.2 ±15.9
Cholestrol (mg/dl)	76.7±14.5	73± 9.3	71.7±18	76.6±14.9
Triglyceride (mg/dl)	86.4±8.3	90.5±5.95	96.5±6.2	91.6 ±10

The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA).

Table 5: Haematological parameters of rats in acute toxicity study.

Parameter	Male		Female	
	Control	Treated	Control	Treated
WBC count (10^3/µl)	10.9 ±4.14	9.4±1.64	9.48±2.38	8.3±0.73
RBC count (10^6/µl)	7.46±1.62	8.29±0.47	7.36±1.55	7.9 ±1.02
Haemoglobin (g%)	13.62±1.44	15.1±0.35	13.9±2.45	14.47±1.2
HCT (%)	45.5 ±9.6	48.4±1.6	44. ±5.92	46.2 ±4.1
MCV (Femto liter)	61.2±3.2	58.54±1.27	61.1±5.8	58.72 ±4.9
MCH (Pico grams)	18.08 ±1.28	18.2 ±0.63	19 ±0.82	18.4±1.3
MCHC (g%)	29.6 ±1.9	31.25±0.35	31.15 ±1.5	31.32±0.42
Reticulocytes (%)	3.13±1.7	4.05±1.5	3.9±0.8	4.75±0.57
Platelet count (103/µl)	1485±192	1467 ±181	1150 ±410	1068±459
Differential Leucocyte Count				
Neutrophils (%)	9.7 ±3.4	9.3±1.3	9.7±3.78	9.56±0.32
Lymphocytes (%)	83.3±5.7	81.7±2.6	77.9 ±8.33	83.6±0.47
Monocytes (%)	5.82 ±2	6.6 ±0.6	9.9±4.6	7.23 ±0.66
Eosinophils (%)	0.76±0.5	0.83±0.24	0.9±0.52	1.3 ±0.85
Basophiles (%)	0.4±0.27	0.27±0.13	2.5±1.21	1.75 ±0.5

The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA)

Table 6: Body weight of rats in sub-acute toxicity.

Group	Body weight (g)				
	0th Day	7th day	14th Day	21th Day	28th day
Male (Treated)	114 ±5.4	132 ±10.3	159±11.9	178±9.0	198±13.0
Male (Control)	144±15.1	165 ±14.1	184±12.4	196±9.6	208 ±7.5
Female (Treated)	108±4.4	131±4.1	151±4.1	161±4.1	171±7.4
Female (Control)	162±13	179±14.7	198±14.4	206±13	218±13

The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA)

Table 7: Average feed and water consumption of rats

	Average feed consumption (g/day)		Average water consumption (ml/day)	
	Male	Female	Male	Female
Control	17.6 ±1.5	18 ±1.87	24.8±1.9	24.2±1.9
Treated	17.6 ±1.1	17.2±0.83	27.8* ±1.3	29.2*±1.3

The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA)

Table 8: Biochemical parameters of rats in sub-acute toxicity study.

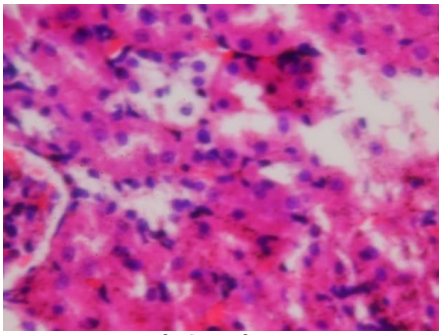
	Male		Female	
Parameter	Control	Treated	Control	Treated
Liver function tests				
ALT (IU/L)	97 ±11	105 ± 18.2	85.8 ±6.8	84.4±11.6
AST (IU/L)	147±15.8	132 ± 18.6	135 ±25.2	211*±48.4
ALP (IU/L)	188.6±33.8	230±56.3	139.3 ±27	130± 26.4
Total Bilirubin (mg/dl)	0.23±0.08	0.32± 0.08	0.25±0.03	0.30 ±0.04
Total Protein (g/dl)	8.07±0.44	8.56 ± 0.46	8.48±0.42	8.7± 0.1
Albumin (g/dl)	4.05±0.21	4.19± 0.30	4.56±0.29	4.3±0.1
Kidney function tests				
Urea (mg/dl)	42.53±5.3	56.2±10.6	53.6± 6.4	47.2± 5.8
Uric acid (mg/dl)	3.78±0.75	4.15±1.7	3.08±0.66	3.3± 0.4
Metabolic function tests				
Glucose (mg/dl)	85±7.8	89.7±9.6	81.4±2.6	91.4±11.9
Cholestrol (mg/dl)	63.7± 8.5	57.7± 5.3	62.6±9.8	62.6±14.8
Triglyceride (mg/dl)	75.2±8.3	46.5*±9.95	83.1±11.1	40.8*±9.5

The values are expressed as mean ± SD. n=5 in each group. *p<0.05 as compared with the controls at the same time (one-way ANOVA).

Table 9: Haematological parameters of rats in sub-acute toxicity study.

Parameter	Male		Female	
	Control	Treated	Control	Treated
WBC count (10^3/µl)	10.53±4.95	13.04±5.03	12.79±1.22	9.68±2.67
RBC count (10^6/µl)	9.42±0.95	9.18±0.76	8.02±1.59	8.65±0.61
Haemoglobin (g%)	17.46±0.92	16.3±1.30	15.53±1.55	15.4±1.40
HCT (%)	54.6±2.6	51.2±3.79	49.0±3.70	48.06±1.9
MCV (Femto liter)	58.2±4.1	55.84±3.03	62.1±7.3	55.68±2.52
MCH (Pico grams)	18.6 ±1.05	17.78±0.79	19.63±1.67	17.84±0.58
MCHC (g%)	31.96±0.46	31.82±0.65	31.6±0.75	32.12±0.93
Reticulocytes (%)	2.12±0.51	2.52±0.71	2.41±0.68	3.03±1.44
Platelet count (10^3/µl)	1434±156	1494±242	1402±156	1453±185
Differential Leucocyte Count				
Neutrophils (%)	9.7±5.02	8.78±3.26	7.2±1.78	9.18±4.45
Lymphocytes (%)	78.6±6.03	85.54±2.8	83.4±2.01	85.14±4.49
Monocytes (%)	10.6±1.8	4.98*±0.63	8.3±3.12	4.76*±1.07
Eosinophils (%)	0.8±0.1	0.68±0.24	0.7±0.55	0.52±0.32
Basophiles (%)	0.26±0.11	0.22±0.04	0.2±0.15	0.4±0.32

The values are expressed as mean ± SD. n=5 in each group. * $p<0.05$ as compared with the controls at the same time (one-way ANOVA)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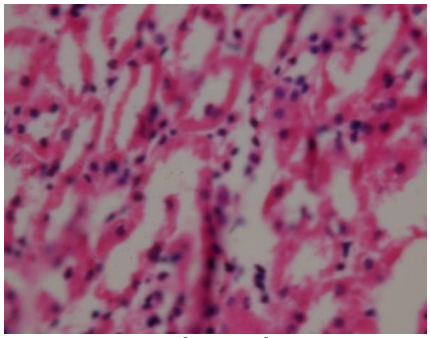
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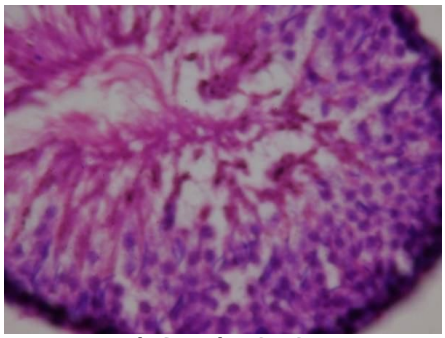
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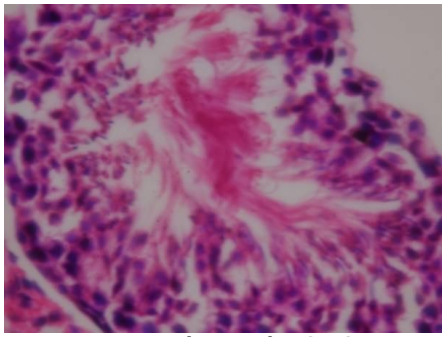
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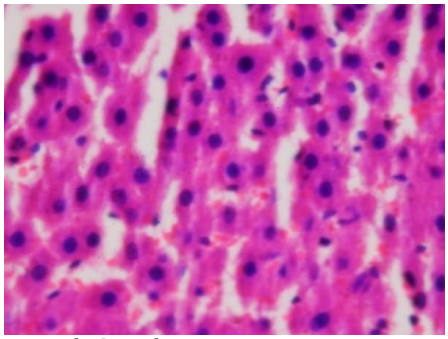
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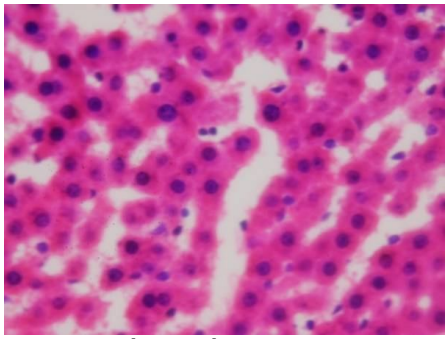
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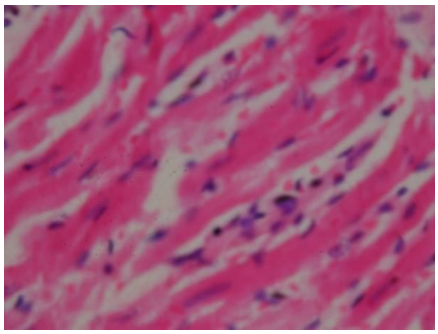
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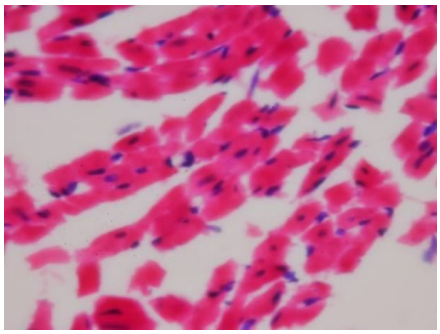
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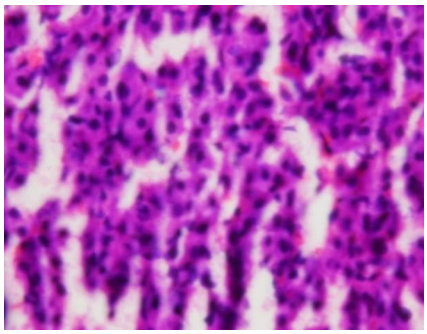
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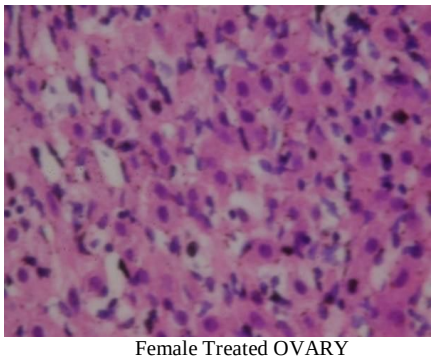
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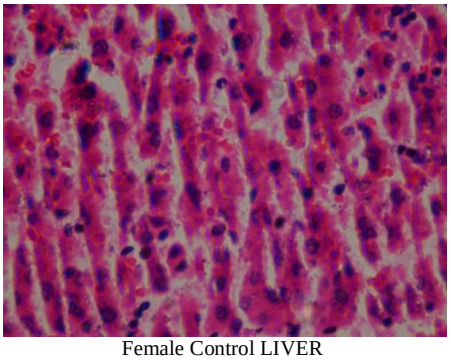
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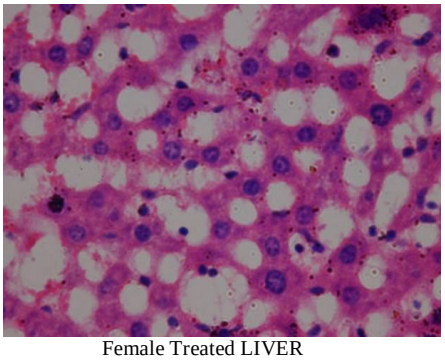
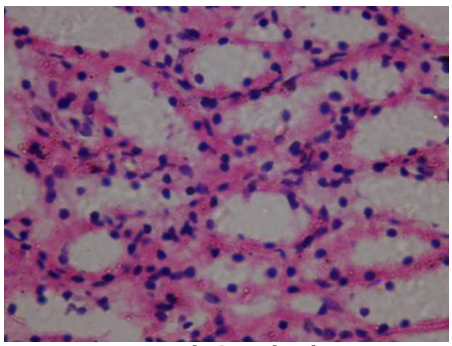
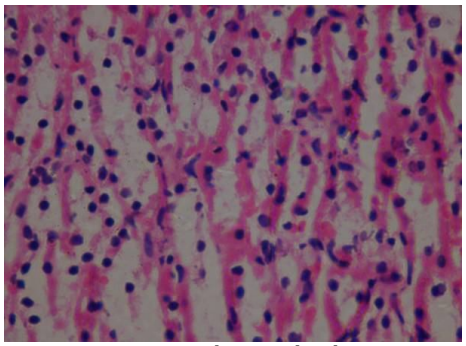
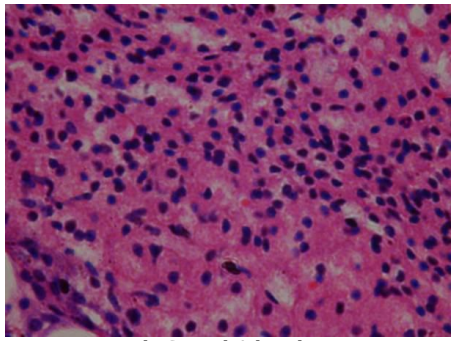
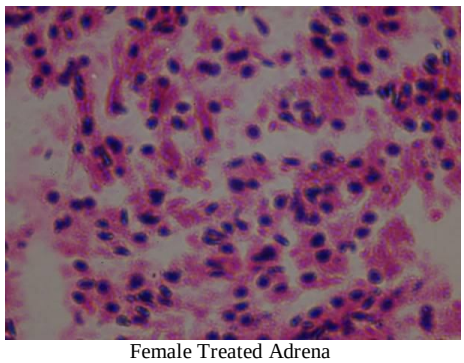
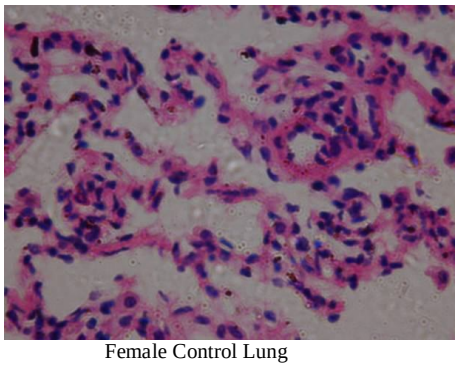

Female Treated LIVER

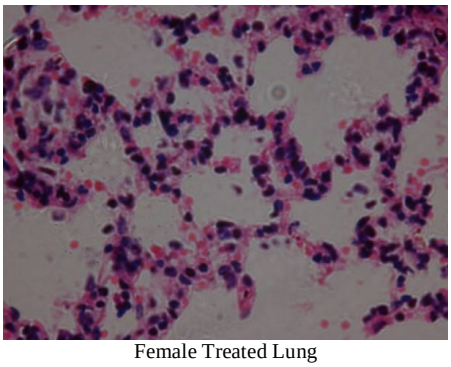

Female Control Kidney


Female Treated Kidney


Female Control Adrenal


Female Treated Adrena


Female Control Lung


Female Treated Lung

Haematology Parameters

In haematological parameters there was a significant change in the percentage of Monocyte cells in both male and female rats of the treated groups.

Examination of Organs and Tissues

Gross

The macroscopic examination of liver, kidney, lung, brain, heart, spleen, testis/ovary and adrenal gland of treated groups did not show any morphological difference when compared with the control group. The shape, size and texture of these collected organs of treated rats were found to be normal.

MICROSCOPIC

The Histopathological examination of treated animals also indicated that there was no damage to the tissue / organs when compared with the control animals as shown bellow.

DISCUSSION AND CONCLUSIONS

The strategy for establishment of safety of a test item depends on demonstration of its adverse effects (toxicity) or no adverse effects (no toxicity) under the conditions of exposure to its high doses to the test animals (Rodents or non-rodents).

Having defined the toxicity (dose, organ, biomarker of the adverse effect), the likelihood of occurrence of the adverse effect is judged in the light of the possible (or intended) exposure levels (or dose levels of exposure) in the human situation, and simultaneously the safe level of exposure is estimated. Initially, these studies for demonstration of toxicity are conducted in rodents, using the limit doses which are based on the technical feasibility as well as scientific utility of the highest single or repeated (or daily) doses of the test item that can be given to the animals. The first study aimed at demonstrating the toxicity of a "single high dose" of a test item is called Acute Toxicity Study, and as per the internationally accepted OECD test guidelines, it

employs the exposure to a single oral dose of 2.0g/kg b.wt. of the test item in the rodent species, followed by an observation period of 14 days.

Next, the study aimed at demonstrating the toxicity of "repeated daily dosing" of a test item is called Subacute Toxicity Study, and as per the internationally accepted OECD test guidelines, it employs the daily exposure to daily oral dosing of 1.0g/kg b.wt. of test item in the rodent species for a period of 28 days. The observation parameters of these studies include a comparative evaluation of general appearance/behaviour, morbidity/mortality, body weights, food/water consumption, and laboratory investigations of haematology, blood biochemistry and histopathology of major organs and tissues in the animals of treated and control groups in each of these studies. The test item "Deedan" is a very effective compound formulation of Unani System of medicine, traditionally used for the treatment of worm infestation. In the clinical setting, one capsule containing 500mg of Deedan is given to adult human subjects twice a day orally with water for 7 days.

Possible toxic effects and the limits of safety of Deedan are not so far studied, therefore the objective of our studies was to investigate the Acute and Sub-acute toxicity of Deedan in young, healthy Albino rats (Wistar Strain) of both the sexes. Herbs and herbal products are globally used by humans and has assumed exponential increase (Brevort, 1998; Bodekar et al., 2005).

However issues related to the safety and side effects of these herbal products in recent times are becoming also more evident. It is a bitter truth that "natural" and "safety" are not synonymous (Martin, 2013). There is a great need that regulatory policies on herbal products and medicine need to be standardised and strengthened globally (WHO, 2004).

Acute toxicity

Toxicology studies are the platform for hazard identification stage of safety assessment (Wallace, 2011). As per OECD guideline 425 the dose 2000 mg / kg body weight is said to be "Unclassified" under the toxicity scale. Hence further study with higher doses was not executed. The acute study of Deedan at the limit dose of 2000 mg/kg body weight did not cause any adverse sign of toxicity therefore it is non toxic and safe in oral formulation. There was no statistically significant difference in the mean body weight, feed and water consumption, haematological and biochemical results between experimental and control groups during the study. Gross examination of organs and tissues (liver, kidney, lung, brain, heart, spleen, testis/ovary and adrenal gland) of treated groups did not show any significant difference when compared with the control group. This study also suggests that the LD50 value of Deedan is greater than 2000 mg/kg body weight of albino rats, since all the rats survived, and there was no sign of abnormalities in their general behaviour throughout the period of 14 days study.

Sub-acute toxicity

Repeated oral administration of Deedan dose 1000mg/kg body weight for 28 days did not cause lethality or any significant sign of toxic effect in male and female rats. There were no significant difference in body weight between experimental and control groups during the study period. The haematological parameters did not show any significant difference expect there is a little decrease in Monocyte cell count in both the male and female treated rats when compared with the control ones. The biochemistry results indicate that the Deedan does not alter the liver enzymes GOT/GPT and ALP, the kidney function tests Blood urea, uric acid were also not affected. The results also indicate that the other biochemical substances such as blood glucose, serum cholesterol were not altered. The results indicate that the drug Deedan is effective in decreasing the Triglyceride concentration in blood. The decrease in serum Triglyceride by Deedan in both sexes of rats is possibly due to the effects of Tukhm-e-Hanzal (*Citrullus colocynthis*) and Turbud (*Operculina turpethum*) as the plant materials of these two herbs have been reported to be hypolipidemic (Rahbar et al., 2010; Veena et al., 2012). Gross and Microscopic examination of organs and tissues (liver, kidney, lung, brain, heart, pancreas, spleen, testis/ovary and adrenal gland) of treated groups did not show any significant difference when compared with the control group. Deedan was found to be free of any toxic effects under the conditions of this study.

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REFERENCES

Razzack MA. Holistic concepts in Unani Medicine. *Hippocratic journal of Unani Medicine*, 2006; 1(2): 17-20.

Debbie S, Ladds G, Duez P, Williamson E, Chan K. Pharmacovigilance of herbal medicine. *Journal of Ethnopharmacology*, 2012; 140 (3):513-518.

Wal P, Wal A, Gupta S, Sharma G, Rai, AK. . Pharmacovigilance of herbal medicine in India. *Journal of Young Pharmacists*, 2011. 3(3): 256-258

CCRUM. National formulary of Unani medicine. 2007. Part II, Vol1.

Capdevila S, Giral M, Ruiz JL, de la Torre, Russell RJ, Kramer K. Acclimatization of rats after ground transportation to a new animal facility. *Laboratory Animals*, 2007; 41(2): 255-261.

OECD. Test no. 425. OECD Guidelines for testing of chemicals. Single dose 14 days oral toxicity study in Rodents, 2008.

OECD. Test no. 407. OECD Guidelines for testing of chemicals. Repeated dose 28 days oral toxicity study in Rodents, 2008.

Brevort P. The booming us botanical market: a new overview. *Herbal Gram*, 1998; 44:33-48.

Bodekar C, Bodekar G, Ong C, Grundy CK, Burford G, Shein K. Who global atlas of traditional, complementary and alternative medicine. Geneva, Switzerland, WHO, 2005.

Martin E. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 2013; 4,177

WHO. WHO guidelines on safety monitoring of herbal medicines in pharmacological systems, 2004.

Wallace HM. Risk perception in toxicology-Part II: Toxicology must be the solution not the problem. *Toxicology Science*, 2011; 121(1):7-11.

Rahbar AR, Nabipour I. The hypolipidemic effect of *Citrullus colocynthis* on patients with hyperlipidemia. *Pakistan journal of biological science*, 2010; 13(24):1202-1207.

Veena S, Manu S. Alterations induced by N-Nitrosodimethylamine and ethanolic root extract of *Operculina turpethum* in serum lipid profile of male albino mice. *Asian journal of pharmaceutical and clinical research*, 2012; 5: 3.

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